CLAIMS

What is claimed is:

1. A method of determining the sequence of a nucleic acid template, said method comprising:

i) generating and redox labeling sets of complementary sequencing fragments of said template where the sets of fragments terminating with the four different bases A, C, G, or T are each label labeled with a redox-active label that has an oxidation state distinct and distinguishable from the redox states of the labels labeling the other sets of fragments;

ii) separating said sequencing fragments;

iii) performing cyclic voltammetry on said sequencing fragments to produce a cyclic voltammogram for the redox-labeled sequencing fragments;

iv) detecting the signal for each redox-active label at a phase angle out of phase with respect to the optimum phase angle for said redox-active label, where a drop-out of signal at said phase angle indicates the presence of said redox-active label.

2. The method of claim 1, wherein said dropout is as compared to the signal present at the phase common signal.

3. The method of claim 1, wherein said fragments are generated with a termination method employing primers, and terminators, and the primers or the terminators are labeled with said redox-active labels.

4. The method of claim 3, wherein said fragments are generated with dideoxy terminators.

5. The method of claim 4, wherein said fragments are generated with dideoxy terminators selected from the group consisting of 2',3'-dideoxyguanosine-5'-triphosphate, 7-deaza-2',3'-dideoxyguanosine-5'-triphosphate, 2',3'-dideoxyadenosine-5'-triphosphate, 2',3'-dideoxythymidine-5'-triphosphate, and 2',3'-dideoxycytidine-5'-triphosphate.

6. The method of claim 1, wherein nucleoside triphosphates used for chain elongation are labeled with said redox-active labels.

7. The method of claim 1, wherein said redox-active labels are independently selected from the group consisting of a porphyrin, an expanded porphyrin, a contracted porphyrin, a metallocene, a linear porphyrin polymer, and a porphyrin array.

8. The method of claim 7, wherein said redox-active labels comprise a ferrocene.

9. The method of claim 8, wherein said ferrocene is selected from the group consisting of an alkyl ferrocene, a ferrocene acetate, a ferrocene carboxylate, and an alkyl ferrocene dimethylcarboxamide.

10. The method of claim 1, wherein said redox-active labels comprise a porphyrinic macrocycle substituted at a β- position or at a *meso*- position.

11. The method of claim 1, wherein said voltammetry is performed at a single electrode.

12. The method of claim 1, wherein said voltammetry utilizes a sinusoidal waveform.

13. The method of claim 1, wherein said cyclic voltammetry comprises converting voltammetric data into a time or frequency domain to provide a frequency spectrum for a redox-active label.

14. The method of claim 12, wherein said cyclic voltammetry comprises converting voltammetric data into a time or frequency domain to provide a frequency spectrum for a redox-active label.

15. The method of claim 13, wherein said converting comprises performing a Fourier transform.

16. The method of claim 13, wherein said cyclic voltammetry comprises selecting voltammetric data at a second or higher harmonic frequency.

17. The method of claim 16, wherein said cyclic voltammetry comprises selecting voltammetric data at a third or higher harmonic frequency.

18. The method of any one of claims 1, 13, or 16, wherein said cyclic voltammetry comprises selecting voltammetric data at a phase angle about 45 degrees to about 90 degrees out of phase with the optimum phase angle for the redox-active label whose presence is to be detected.

19. The method of claim 18 wherein said cyclic voltammetry comprises selecting voltammetric data detecting at a phase angle closest to 90 degrees out of phase with the optimum phase angle for the redox-active label whose presence is to be detected.

20. The method of claim 1, wherein separating said sequencing fragments comprises electrophoretically separating said sequencing fragments.

21. The method of claim 1, wherein separating said sequencing fragments comprises chromatographically separating said sequencing fragments.

22. A chain-termination type nucleic acid sequencing method, said method comprising:

i) providing a template nucleic acid;

ii) annealing an oligonucleotide primer to a portion of said template nucleic acid thereby forming a primer-template hybrid;

iii) adding a primer-extension reagent to the primer-template hybrid for extending the primer and forming a primer extension product, the primer extension reagent comprising nucleoside triphosphates; and

iv) adding a terminator to the primer-template hybrid for causing specific termination of the primer extension and formation of a plurality of primer extension products where said terminator or said oligonucleotide primer is labeled with one of four redox-active tags where said redox-active tags have different and distinguishable oxidation states;

v) separating said primer extension products; and

vi) detecting the signal for each redox-active label at a phase angle out of phase with the optimum phase angle for said redox-active label, where a drop-out of signal at said phase angle indicates the presence of said redox-active label.

23. A method of detecting a tagged analyte, said method comprising:

i) providing at least two species of tagged analyte

ii) performing cyclic voltammetry on said tagged analytes to produce a cyclic voltammogram for said tagged analytes;

iii) detecting the signal for a redox-active label at a phase angle out of phase with the optimum phase angle for said redox-active label, where a drop-out of signal at said phase angle indicates the presence of said redox-active label.

24. The method of claim 23, wherein said providing comprises providing at least four species of tagged analyte where each species of tagged analyte is tagged with a redox-active label where the redox-active label attached to each species has an oxidation state different and distinguishable from the oxidation states of the redox-active labels attached to the other species of tagged analyte.

25. The method of claim 23, wherein said redox-active label is selected from the group consisting of a porphyrinic macrocycle, a metallocene, a linear polyene, a cyclic polyene, a heteroatom-substituted linear polyene, a heteroatom-substituted cyclic polyene, a tetrathiafulvalene, a tetraselenafulvalene, a metal coordination complex, a buckyball, a triarylamine, a 1,4-phenylenediamine, a xanthene, a flavin, a phenazine, a phenothiazine, an acridine, a quinoline, a 2,2'-bipyridyl, a 4,4'-bipyridyl, a tetrathiotetracene, and a peri-bridged naphthalene dichalcogenide.

26. The method of claim 23, wherein said redox-active label is selected from the group consisting of a porphyrin, an expanded porphyrin, a contracted porphyrin, a metallocene, a linear porphyrin polymer, and a porphyrin array.

27. The method of claim 26, wherein said redox-active labels comprise a ferrocene.

28. The method of claim 27, wherein said ferrocene is selected from the group consisting of an alkyl ferrocene, a ferrocene acetate, a ferrocene carboxylate, and an alkyl ferrocene dimethylcarboxamide.

29. The method of claim 23, wherein said redox-active label comprises a porphyrinic macrocycle substituted at a β- position or at a *meso*- position.

30. The method of claim 23, wherein said voltammetry is performed at a single electrode.

31. The method of claim 23, wherein said cyclic voltammetry utilizes a sinusoidal excitation waveform.

32. The method of claim 23, wherein said cyclic voltammetry comprises converting voltammetric data into a time or frequency domain to provide a frequency spectrum for a redox-active label.

33. The method of claim 32, wherein said converting comprises performing a Fourier transform.

34. The method of claim 32, wherein said cyclic voltammetry comprises selecting voltammetric data at a second or higher harmonic frequency.

35. The method of claim 44, wherein said cyclic voltammetry comprises selecting voltammetric data at a third or higher harmonic frequency.

36. The method of any one of claims 23, 32, 34, or 35 wherein said cyclic voltammetry comprises selecting voltammetric data at a phase angle about 45 degrees to about 90 degrees out of phase with the optimum phase angle for the redox-active label that is to be detected.

37. The method of claim 36 wherein said cyclic voltammetry comprises selecting voltammetric data detecting at a phase angle about 90 degrees out of phase with the optimum phase angle of the redox-active label that is to be detected.

38. The method of claim 23, wherein said analytes are selected from the group consisting of nucleic acids, proteins, and antibodies.

39. The method of claim 23, wherein said redox-active label is attached to a chain terminator.

40. The method of claim 23, wherein said redox-active label is attached to a nucleic acid.

41. The method of claim 23, wherein said analytes are electrophoretically separated nucleic acids.

42. The method of claim 23, wherein said analytes are chromatographically separated nucleic acids.

43. The method of claim 23, wherein said providing comprises providing four species of tagged analyte where each species of tagged analyte is tagged with one of four different and distinguishable redox-active label.

44. A method of selective electrochemical detection of analytes in a complex mixture of analytes, said method comprising:

i) labeling each analyte in the mixture with a redox label that generates an electrochemical signal that is different from the labels attached to other analytes in said mixture where said labeling provides labeled analytes;

ii) performing cyclic voltammetry on said labeled analytes to produce a cyclic voltammogram for said labeled analytes;

iii) detecting the signal for a redox-active label at a phase angle out of phase with the optimum phase angle for said redox-active label, where a drop-out of signal at said phase angle indicates the presence of said redox-active label.

45. A computer-readable medium that can be used for directing an apparatus to detect and distinguish a plurality of redox-active tags where said redox-active tags have different and distinguishable oxidation states, said computer readable medium comprising;

computer readable program code for directing a potentiostat in a cyclic voltammetric measurement to produce a cyclic voltammogram of said redox-active tags;

computer readable program code for detecting the signal for each redox-active label at a phase angle out of phase with the optimum phase angle for said redox-active label, where a drop-out of signal at said phase angle indicates the presence or amount of said redox-active label.

46. The computer readable medium of claim 45, wherein said plurality of redox-active tags comprises four redox-active tags.

47. The computer readable medium of claim 45, wherein said cyclic voltammetric measurement is performed at a single electrode.

48. The computer readable medium of claim 45, wherein said cyclic voltammetric measurement is sinusoidal voltammetry.

49. The computer readable medium of claim 45, wherein said code for detecting the signal comprises code for converting voltammetric data into a time or frequency domain to provide a frequency spectrum for a redox-active label.

50. The computer readable medium of claim 49, wherein said converting comprises performing a Fourier transform.

51. The computer readable medium of claim 45, wherein said code for detecting the signal comprises code for selecting voltammetric data at a second or higher harmonic frequency.

52. The computer readable medium of claim 51, wherein said code for detecting the signal comprises code for selecting voltammetric data at a third or higher harmonic frequency.

53. The computer readable medium of any one of claims 46, 51, or 52, wherein said code for detecting the signal comprises code for selecting voltammetric data at a phase angle about 45 degrees to about 90 degrees out of phase with the optimum phase angle for said redox-active label.

54. The computer readable medium of claim 53, wherein said code for detecting the signal comprises code for selecting voltammetric data detecting at a phase angle about 90 degrees out of phase with the optimum phase angle for said redox-active label.

55. The computer readable medium of claim 45, wherein said computer readable medium is selected from the group consisting of a magnetic disk, an optical disk, and a chip.

56. The computer readable medium of claim 45, wherein said computer readable medium is a component of a nucleic acid sequencer.

57. A computer-readable storage medium storing program code for causing a computer to detect and distinguish a plurality of redox-active tags where said redox-active tags have different and distinguishable oxidation states, said computer readable medium comprising program code directing a computer to:

detect the signal for each redox-active label at a phase angle out of phase with the optimum phase angle for said redox-active label, where a drop-out of signal at said phase angle indicates the presence or amount of said redox-active label.

58. The computer readable storage medium of claim 57, wherein said computer readable medium further comprises program code for directing a potentiostat in a cyclic voltammetric measurement to produce a cyclic voltogram of said redox-active tags.

59. A kit for sequencing a nucleic acid, said kit comprising:

four redox-active tags wherein said redox active tags have different and distinguishable oxidation states; and

instructional materials teaching the detection of the signal for each redox-active label at a phase angle out of phase with the optimum phase angle for said redox-active label, where a drop-out of signal at said phase angle indicates the presence or amount of said redox-active label.

60. The kit of claim 59, wherein said redox-active labels are attached to elongation terminators.

61. The kit of claim 60, wherein said elongation terminators are dideoxy elongation terminators.

62. A kit for sequencing a nucleic acid, said kit comprising:

a plurality of redox-active tags where said redox active tags have different and distinguishable oxidation states; and

a computer readable medium of claim 45.

63. The kit of claim 62, wherein said kit comprises four or more redox-active labels.

64. A kit for sequencing a nucleic acid, said kit comprising:

a plurality of redox-active tags where said redox active tags have different and distinguishable oxidation states; and

a computer readable medium of claim 57.

65. The kit of claim 64, wherein said kit comprises four or more redox-active labels.

66. In a computer system containing stored software programs, a method of detecting a tagged analyte from a plurality of tagged analytes, said method comprising:

ii) performing cyclic voltammetry on a plurality of tagged analytes where each species of tagged analyte is tagged with a redox-active label where the redox-active label attached to each species has an oxidation state different and distinguishable from the oxidation states of the redox-active labels attached to the other species of tagged analyte, and said voltammetry produces a cyclic voltogram for said tagged analytes, wherein said cyclic voltammetry is performed by a potentiostat under control of said computer system; and

ii) detecting the signal for a redox-active label at a phase angle out of phase with the optimum phase angle for said redox-active label, where a drop-out of signal at said phase angle indicates the presence of said redox-active label, wherein said detecting comprises analysis of said voltogram by said computer system.

67. A computer system, for detecting a redox active tag among a plurality of redox active tags, said computer system comprising:

a memory configured to store software programs;

a data acquisition and control interface for acquiring data from a potentiostat; and

a computer readable medium comprising computer readable program code for directing said potentiostat in a cyclic voltammetric measurement to produce a cyclic voltammogram of said redox-active tags; and

computer readable program code for detecting the signal for each redox-active label at a phase angle out of phase with the optimum phase angle for said redox-active label, where a drop-out of signal at said phase angle indicates the presence or amount of said redox-active label.

5